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EDITORIAL

Do oxidized lipoproteins contribute to glomerulosclerosis?

Lipoproteins have long been associated with the development of atherosclerosis. As atherosclerotic processes were thought not to occur in capillaries, for a long time lipids were not considered to be pathogenetic factors in glomerulosclerosis. Based on early observations of Virchow, Kimmelstiel, Wilson and French, several investigators have recently gathered experimental evidence in rats and other small mammals that hyperlipoproteinemia and hypercholesterolemia can induce and contribute to fibrosis of glomerular capillaries [reviewed in 1]. Diamond and Karnovsky coined the term "capillary atherosclerosis" [2]. There are indications that lipid-modulated glomerular damage also occurs in humans [1]. Patients with lecithin-cholesterol acyltransferase (LCAT)-deficiency have pronounced dyslipidemia with lipid deposits in glomeruli and as a consequence develop severe glomerulosclerosis. Apolipoproteins apoB-100 and apoE and LDL-receptor proteins have been detected in glomeruli of patients with different glomerular diseases [3, 4].

To gain insights into lipoprotein-associated glomerulosclerosis, the effects of usual lipoproteins and lipoproteins with abnormal composition (such as lipoproteins of patients with LCAT-deficiency) on mesangial cells and podocytes have been investigated. Native lipoproteins stimulated cell proliferation, the early response genes involved in DNA synthesis, the synthesis of cytokines/growth factors (for example, M-CSF, PDGF, MCP-1) and of matrix proteins such as collagen and fibronectin [1, 5]. As oxidatively modified lipoproteins have been strongly implicated in the genesis of atherosclerosis [6], it was logical to also analyze the effects of oxidatively modified lipoproteins on glomerular cells in culture. Oxidatively modified lipoproteins exerted the same effects as native lipoproteins when they were only minimally modified, that is, still able to specifically bind to the apoB/E receptor. Oxidatively, greatly modified lipoproteins that bound to scavenger receptors were antiproliferative and induced apoptosis. Their effects on the synthesis of growth factors and matrix proteins have been discussed controversially [7]. Oxidatively modified lipoproteins by themselves exert chemotactic effects on granulocytes, T cells and monocytes [8].

In rats with focal segmental sclerosis oxidatively modified lipoproteins were detected in the glomerular scars [9].

Using immunohistology and Western blot Lee and Kim in this issue now demonstrate that oxidatively modified (lipo)proteins can be found in human glomeruli with immune complex and degenerative diseases often colocalizing with apolipoprotein B-100 [10]. The authors have studied a large number of biopsies to demonstrate oxidatively modified lipoproteins in segmental scars and mesangium. Interestingly, oxidatively modified lipoproteins were not only found in glomerular disease characterized by an influx of polymorphonuclear leukocytes and monocytes/macrophages (such as necrotizing glomerulonephritis, membranoproliferative glomerulonephritis), but also in a rather high percentage (>25%) of patients with degenerative disease (nephrosclerosis). It is astounding that the authors could only detect one (kidney tissue) and two (ox-LDL) modified proteins by Western blot. Oxidative modification, especially that catalyzed by Cu^{++} *in vitro* usually leads to an irregular fragmentation of apoB-100 and other fortuitously modified proteins resulting in a smear on Western blots. The existence of oxidatively modified lipoproteins did not correlate closely with hypercholesterolemia and proteinuria.

This is in contrast to elegant studies in Heymann nephritis, the experimental rat model of human membranous glomerulopathy. Oxidative modification of the noncollagenous domain of collagen type IV in the glomerular basement membrane, detected with specific antibodies to malondialdehyde containing epitopes, was correlated with proteinuria [11]. Also, in these experiments apolipoprotein E and oxidatively modified epitopes were detected in the subepithelial immune deposits in the glomerular basement membrane. Antioxidants significantly decreased proteinuria [12]. Thus, oxidative modifications within the glomerular basement membrane can cause functional defects.

It is probable that other oxidative products will be found in human glomeruli and the percentage of glomeruli with oxidative modifications may well increase significantly in different glomerular diseases.

Oxidative modifications are generated by a multitude of chemical reactions resulting in a complex array of different oxidative epitope modifications [13]. Lee and Kim have identified malondialdehyde modified epitopes. Evidence has been provided that oxidative modifications by hypochlorous acid can be found in glomeruli of patients with nephrosclerosis [14]. Discrete deposits of hypochlorous acid modified protein were also demonstrated in the basement membrane, in mesangial cells and in podocytes in human membranous glomerulonephritis [15].

Although Lee and Kim do not specifically address their

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finding of oxidatively modified proteins in basement membranes of regular glomeruli, these results may be relevant for the understanding of glomerular (re)modeling. Apparently, in normal kidney reactive oxygen species (ROS) and reactive oxygen compounds are generated in sufficient quantities to lead to oxidative modifications of glomerular basement membrane proteins. Alternatively, oxidatively modified proteins may be filtered into and retained by the glomerular basement membrane. It is not yet clear whether these slight oxidative modifications have functional consequences.

Lee and Kim tried in vain to associate the localization of malondialdehyde modified proteins with the electron-transporting component of NADPH oxidase. In contrast, cytochrome b558 mRNA and protein expression has been found to rise dramatically in podocytes of glomeruli with Heymann nephritis and with oxidative modification of glomerular basement membrane structures. Shah and thereafter many other groups could show that glomeruli, mesangial cells and visceral epithelial cells can generate ROS [16]. In hypercholesterolemia isolated rat glomeruli increase their basal and phorbol myristate acetate stimulated ROS synthesis. Infiltrating monocytes/macrophages probably participate significantly in this rise of ROS generation in hyperlipidemia. Monocytes/macrophages are required for lipid-induced glomerulosclerosis to occur [17]. The new finding by Lee and Kim of only tiny amounts of NADPH oxidase proteins in glomerular endothelial cells and podocytes seems to underscore the quintessential role of monocytes in oxidative processes. Recent studies in the rat have not only demonstrated an increase in NADH/NADPH oxidase activity but also in xanthine oxidase activities in hyperlipidemia (Scheuer H, Gwinner W, Gröne HJ, manuscript in preparation). It remains still to be elucidated (1) which enzyme, nonenzymatic pathway or dysbalance of oxidant/antioxidant enzymes contributes most to the generation of oxygen species and oxygen mediated modification, and (2) which mechanisms initiate these oxidative pathways in hyperlipidemia.

In rat experiments a rise in glomerular ROS was already found in hypercholesterolemia well before lipid deposits and oxidatively modified lipoproteins could be localized. This was also true for tubulointerstitial damage that could be associated with hyperlipoproteinemia. Oxidatively modified lipoproteins in glomeruli thus seem to represent rather late and not obligatory phenomena of lipoprotein-induced glomerular damage. Nevertheless, these oxidized proteins may amplify the sclerotic process by their own inflammatory effects. The overall low percentage of biopsies with oxidatively modified lipoproteins may be due to a rather efficient clearance of oxidation products, and importantly has to be viewed under the aspect that apparently weak immunohistologic stains for oxidized protein were not included in the final evaluation by Lee and Kim.

In summary, the data of the animal and cell culture experiments and the descriptive data in human biopsies indeed suggest that oxidatively modified lipoproteins may contribute to the pathogenesis of glomerulosclerosis. It seems that the concept for irreversible glomerular damage has to incorporate lipid-induced oxidative damage and oxidative modifications of (lipo)proteins as a modulating factor.

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